1-1-1974

Artificial insemination in sheep.

E. K. Inskeep

J. T. Stevens

J. B. Peters

Lance Kauff

ARTIFICIAL INSEMINATION IN SHEEP

Bulletin 629
March 1974

West Virginia University
Agricultural Experiment Station

[Blank Page in Original Bulletin]

ARTIFICIAL INSEMINATION IN SHEEP

PART 1
Artificial Insemination and Preservation of Ram Semen

PART 2
Effect of β-amylase and Time of Insemination on Conception Rates of Ewes Inseminated Artificially

PART 3
Effect of Time of Insemination on Conception Rates of Ewes Bred with Fresh, Undiluted Semen

West Virginia University • Agricultural Experiment Station

EDITOR'S NOTE

Artificial Insemination in Sheep is written in three parts. The complete report covers research conducted at the West Virginia University Agricultural Experiment Station on artificial insemination in sheep since 1965. Review of literature and extensive bibliographies are included. Part 3 carries a report of an experiment conducted on farms cooperating with the University in the Allegheny Highlands Project in Randolph and Upshur counties.

The three parts of this bulletin and authors are:

Part 1 — Artificial Insemination and Preservation of Ram Semen — by E. K. Inskeep, animal scientist.

Part 2 — Effect of β-amylase and Time of Insemination on Conception Rates of Ewes Inseminated Artificially — by J. T. Stevens, formerly graduate research assistant in animal science, and E. K. Inskeep.

Part 3 — Effect of Time of Insemination on Conception Rates of Ewes Bred with Fresh, Undiluted Semen — by J. B. Peters, associate animal scientist with the Allegheny Highlands Project, Elkins; Lance Kauf, research assistant in animal science with the Allegheny Highlands Project; and E. K. Inskeep. — JL.

WEST VIRGINIA UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
COLLEGE OF AGRICULTURE AND FORESTRY
R. S. DUNBAR, JR., DIRECTOR
MORGANTOWN

CONTENTS

[Blank Page in Original Bulletin]

PART 1

ARTIFICIAL INSEMINATION AND PRESERVATION OF RAM SEMEN

E. K. Inskeep

The use of artificial insemination (AI) in sheep has been confined mainly to the Soviet Union, eastern and central Europe, and certain areas in South America. It has been tried experimentally in other areas, but high labor costs, lack of identification of superior sires and factors such as small flocks or limited facilities for handling the animals have kept interest in AI low in western countries and even in Australia and New Zealand (Emmens and Robinson, 1962; Lunca, 1964).

Much of the literature on field trials of AI fails to present details on such items as number and timing of inseminations, the site at which the semen was placed within the reproductive tract, concentration of spermatozoa and initial quality of the semen studied. Thus it often has been difficult to repeat earlier work in which some success had been claimed. Nevertheless, the reviews by Terrill (1960) and by Emmens and Robinson (1962) provide a great deal of practical information. These and partial reviews by Rowson (1959), Almquist (1959) and Lunca (1964) have been able to point to certain general principles and procedures which have been valuable. The present discussion will be limited mainly to the conclusions drawn by these reviewers and to work since 1960.

A single ejaculate of ram semen varies in volume from 0.3 to 2 ml. and usually contains 1 to 5 billion sperm cells per ml. (Salamon, 1964; Mann, 1964). Volume and cell concentration declined with successive ejaculates within a given day and with successive days in studies by Salamon (1964) with Merino rams. The major ions present in ram semen are sodium, chloride, potassium, calcium and magnesium. Mann (1964) found 328 mg. of phosphorus and 875 mg. of nitrogen per 100 ml. semen. The major sugar in ram semen is fructose. Also present are lactic and citric acids in considerable amounts and small amounts of ascorbic acid. Carbon dioxide content is reported by Mann (1964) as 16 ml. per 100 ml. semen.

There has been a considerable effort in recent years, particularly by workers at the University of Sydney, Australia, to characterize the metabolic patterns of ram sperm and to determine the effects of various sugars, alcohols and ions on respiration and motility of ram sperm under laboratory conditions. This basic information should be useful in formulation of suitable diluents for storage of ram semen.

Efforts are being made to use AI in conjuction with control of ovulation by progestogen treatment (Robinson, 1956, 1958, 1967, 1970; Allison and Robinson, 1970; Lightfoot and Salamon, 1970; Quinlivan, 1970; Dziuk *et al.*, 1972). It is in combination that ovulation control and AI may make a contribution to the sheep industry in the United States since labor costs of artificial insemination are prohibitive under natural conditions and ram costs may be prohibitive to natural insemination in large flocks in the case of controlled estrus.

For the purposes of the following discussion it is assumed that rams and ewes which are fertile with natural mating are available. With this assumption, one can consider the special requirements that are peculiar to AI. These will include: (1) collection of semen, (2) number of spermatozoa and volume of inseminating fluid required, (3) dilution and storage of semen, (4) timing of insemination relative to detection of estrus or withdrawal of progestogen treatment, (5) site of deposition of semen, and (6) development of equipment for increasing the efficiency of insemination and for reducing handling stress and labor costs.

METHOD OF COLLECTION

In current practice, two methods are used for collection of ram semen, the artificial vagina and the electro-ejaculator, usually with a bipolar or multipolar rectal electrode. Each method has advantages and disadvantages. As pointed out by Emmens and Robinson (1962) the electro-ejaculator may be used on rams with no training or physically-disabled rams. However, some rams do not respond well to the electrical stimulus, especially if a second or third collection is desired. Furthermore, there is some danger of contamination of the semen sample with urine. Experience has indicated that the degree of stimulation required to obtain ejaculation, the volume of ejaculate and the number of sperm obtained vary considerably from day to day and ram to ram. These variations and the problem of contamination with urine often make it impossible to follow a planned schedule of collecting experimental semen samples and mitigate against the use of electro-ejaculation in a practical AI program.

Most workers have obtained a greater volume of semen but a lower concentration of sperm with electro-ejaculation than with the artificial vagina (Emmens and Robinson, 1962; Mattner and Voglmayr, 1962; Salamon and

Morrant, 1963). Greater concentrations of sodium and potassium were found in both the sperm and seminal plasma obtained by electro-ejaculation (Quinn and White, 1966). The increased amount of seminal plasma and some characteristic which was apparently peculiar to the seminal plasma obtained by electro-ejaculation reduced the resistance of the sperm to cold shock and to deep freezing (Quinn *et al.*, 1968a).

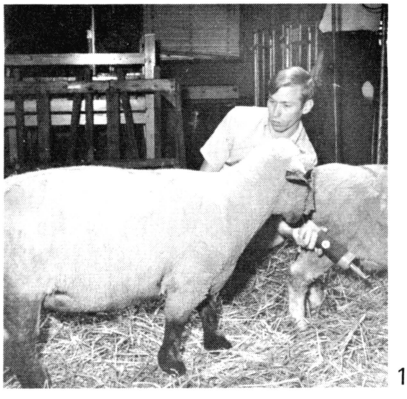
Limited comparisons of fertility have shown little difference between semen samples collected by artificial vagina and electro-ejaculator. In one study, Salamon and Morrant (1963) compared two methods of AI in sheep, one in which semen was collected by artificial vagina and one in which semen was collected by electro-ejaculation. Conception rate at first service was 17 percentage points higher with the method which used semen collected by artificial vagina. They attributed most of this difference to the fact that sperm concentration of the semen was twice as great with the artificial vagina as with the electro-ejaculator but this conclusion may not be justified since several other factors were involved in their experiment. Lapwood *et al.* (1972) found no difference in fertility between semen collected by artificial vagina and that obtained by electro-ejaculation.

On the basis of the work reported to date one must conclude that the artificial vagina is the preferred method for collection of ram semen for artificial insemination.

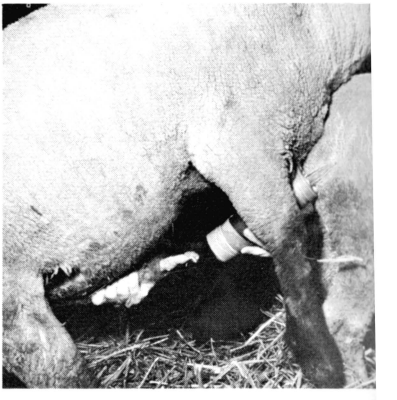
Most rams can be trained for collection by the artificial vagina quite readily with a teaser ewe or ewes that are in heat (Emmens and Robinson, 1962). The teaser ewe is prepared by ovariectomy and treatment with an estrogen, for example, stilbestrol pellets implanted subcutaneously (Rowson, 1959). The casing of the artificial vagina is filled with water at about 41 to 42^{o} C. The recommended pressure is 40 to 60 mm. of mercury. Both temperature and pressure can be judged readily with only limited experience. The inner sleeve is kept relatively short to minimize loss of semen during collection. Once the rams have been trained, the method is easy and quick. Salamon (1964) reported an average interval from release of the ram to ejaculation of only 50 seconds for 130 collections from each of five trained Merino rams. Collection from a trained ram is illustrated in Figure. 1.

NUMBERS OF SPERM AND VOLUME OF INSEMINATING FLUID REQUIRED

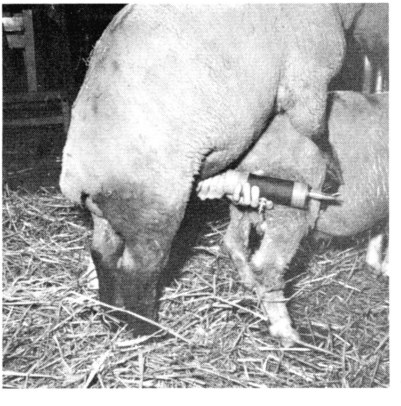
The number of sperm required for insemination will vary with the percentage of live, motile cells in the sample and with the site and time of insemination. Some success has been reported with as few as 5 million sperm (Terrill, 1960). Emmens and Robinson (1962) concluded that a dose of 50 to



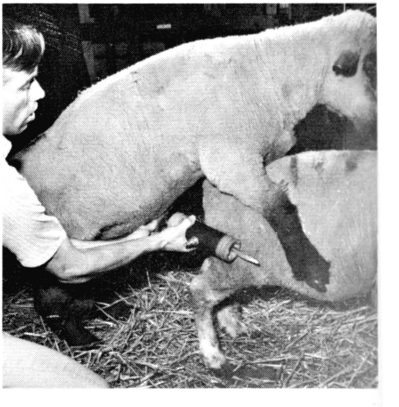
1.



2.

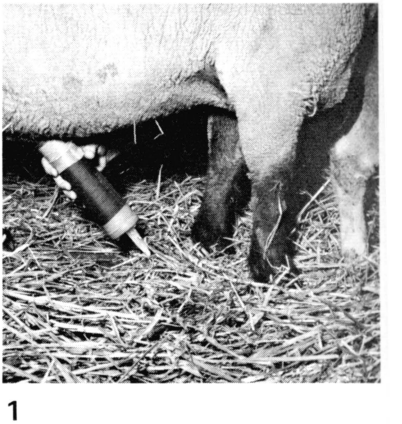


3.



4.

5.



6.

FIGURE 1

Collection of semen from a trained ram by artificial vagina. 1. Ram approaching estrogen-treated, ovariectomized ewe. 2. Mounting and extrusion of penis as operator grasps sheath to guide penis into artificial vagina. 3. Intromission and ejaculation. 4. Withdrawal and dismounting. 5. Semen sample. 6. Well-trained ram may ejaculate without mounting ewe.

150 million sperm in 0.05 to 0.10 ml. of semen, undiluted or diluted, should yield a conception rate of 55 to 60 percent if deposited into the cervix. Salamon (1962) inseminated with 0.1 ml. fresh semen diluted 1:2 with heated, whole cows' milk using alternate ejaculates from Merino rams which were collected 11 times daily for five days. He found that the decline in fertility with successive ejaculates could be accounted for entirely by the decline in sperm numbers. He concluded that at least 120 to 125 million normal sperm were needed for maximum fertility from fresh semen and that each 25 million less down to a dose of 25 million would reduce lambing rate by about 13 percent.

More recently, Quinlivan and Robinson (1969) and Hawk and Conley (1971) have determined that less than 1 percent of sperm used for AI remain in the reproductive tract for 24 hours. Progestin treatment interferes with sperm transport (Quinlivan and Robinson, 1967, 1969) and increases sperm breakage in the vagina when administered by sponges (Hawk, 1972). However, Quinlivan (1970) found no difference in fertilization rate between Cronolone-treated and untreated ewes during the breeding season with sperm numbers ranging from 100 to 1,500 million in undiluted semen. In summer, fertilization rate was equal in progestin-treated and in teased ewes inseminated with 300 million sperm, but increased linearly from 44 to 76 percent as sperm numbers were increased from 300 to 1,500 million. In preliminary work in Israel with Awassi ewes, Schindler *et al.* (1972) have obtained about equal conception rates with 120, 80 or 40 million sperm in reconstituted skim milk. One would conclude that 100 to 150 million cells are adequate during the breeding season, but that up to 1,500 million could be advantageous during anestrus.

The usual practice in most studies utilizing AI as a tool has been to use 0.1 ml. of fresh semen, either undiluted or diluted 1:1 or 1:2 with such diluents as milk or egg yolk-citrate. Volumes greater than 0.4 ml. are seldom used, but Tjupic (1959) has reported lambing rates of 47 to 56 percent from vaginal insemination of 0.5 or 1.0 ml. semen diluted 1:10 or 1:20 and 70 to 72 percent from 1.0 ml. diluted 1:10, or 0.2 ml. diluted 1:2 or 1:4. Similarly, French workers (Dauzier *et al.* 1954) obtained lambing rates of 50 to 83 percent in limited numbers of ewes with 1.0 ml. of semen diluted 1:30 or 1:40.

In case of stored semen, a standard dose for insemination has not been established. The buffering capacity of diluents commonly used for room temperature storage of bull sperm is such that sperm concentrations would have to be much lower than 50 million per 0.1 ml. which Emmens and Robinson (1962) recommended for reasonable fertility. Thus, larger volumes of inseminating fluid would be required to carry the same number of sperm.

The effect of volume of fluid at a constant total number of cells has been studied by Entwistle and Martin (1972) at sperm numbers per insemination of 50 and 100 million. Fertility did not differ with volumes ranging from 31 to 200 microliters or between the two levels of sperm numbers in a buffered glucose diluent.

DILUTION AND STORAGE

Dilution of ram semen, even though mild in degree, usually can be demonstrated to have negative effects on the sperm cells. Percent motility is reduced, regardless of diluent, particularly as dilution rate is increased. Greater dilution accounts in part for the lower resistance to cold shock of sperm collected by electro-ejaculation (Quinn *et al.,* 1968a). In this case the diluent is seminal plasma. Washing to remove seminal plasma also exerts a dilution effect, but a concentration of 0.004 M potassium chloride protects against this effect, suggesting that loss of potassium may be important (Emmens and Robinson, 1962). Recently Lapwood *et al.* (1972) have shown that fertility was lower with diluted than with undiluted semen even though the same numbers of sperm were used.

Research workers have diluted ram semen in nearly every imaginable kind of medium from cows' milk to coconut milk to tomato juice to Bulgarian mineral waters. Egg yolk-citrate, egg yolk-phosphate and cows' milk have been studied most extensively. The yolk-citrate diluents have contained many different concentrations of both yolk and sodium citrate along with several different sugars or alcohols as additives and various antibiotics in some cases. It would be impossible to review each individual study here but a few of the more promising methods will be considered.

Fresh semen

Emmens and Robinson (1962) point out that the egg yolk-citrate and phosphate diluents were developed initially for bull semen rather than for the ram. Nevertheless, yolk-citrate-glucose diluents, usually with penicillin and streptomycin added, appear to be used extensively for diluting ram semen in the Soviet Union, Rumania and Poland (Lunca, 1964). Dilution rates are no higher than 1:6 and the reported conception rates in these countries to a single insemination range from 50 to 90 percent.

Heated milk of both cows and ewes has been tested as a diluent for ram semen. Cows' milk was superior to ewes' milk in trials by Salamon and Robinson (1962a). Dilution of semen 1:4 in either whole or skimmed, heated cows' milk followed by insemination at a dose of 0.3 ml., did not depress fertility from that found with 0.1 ml. undiluted semen. Ewes' and cows' milk were about equal in *in vitro* studies by Jones (1969b). Comparisons of milk with yolk-citrate and yolk-phosphate diluents have shown the milk to be equal or slightly superior. Heated whole milk or reconstituted whole milk powder were chosen for fresh semen in several studies by Robinson and Salamon and their colleagues in Australia. Martin and his students have compared milk and synthetic diluents in several studies (Martin, 1968). Lunca (1964) also cites the use of milk diluents in several eastern and central European countries where conception rates of 64 to

80 percent were reported. Dilution rates as great as 1:5 are apparently satisfactory.

Short-term storage

Motility and the ability to effect fertilization have been lost rapidly in most cases when ram sperm were stored at temperatures of 0 to 12° C in the standard diluents discussed above. In fact, Entwistle and Martin (1972) found that fertility of semen in several diluents fell significantly as the interval from collection to insemination increased to one hour. Roberts and Houlahan (1961) obtained only 39 percent conception in ewes when semen was diluted 1:2 in the standard Russian egg-yolk citrate diluent and stored for 24 hours as compared to 63 percent conception in ewes bred with fresh semen. There are occasional reports of reasonable fertility after storage for 24 to 48 hours. Perhaps the study by Salamon and Robinson (1962b) illustrates reasonably typical results. They compared an egg yolk-citrate-glucose diluent and whole cows' milk at a dilution rate of 1:2 with or without 1,000 i.u. penicillin per ml. Semen was taken from two rams and stored at 0 to 5° C for up to 72 hours. Milk was inferior to the yolk-citrate diluent without the addition of an antibiotic, but with penicillin present, the diluents did not differ. Fertility declined with storage of the semen from 60 percent with fresh semen to 50 percent at 24 hours, 30 percent at 48 hours and 20 percent at 72 hours. These values were based on 80 ewes per group. In this study there was a 9 percent difference in the fertility of semen from the two rams. Other studies have indicated that ram differences are to be expected.

Wiggan and Clark (1967) inseminated three groups of 18 ewes each with semen stored in a modified Minnesota G0 diluent for 12, 36, or 60 hours at 5° C. Dilution rate was 1:5 and ewes were inseminated each time they were found in heat at twice daily heat checks. Conception rates to first service were 83, 67 and 22 percent, respectively.

The use of CO_2 as a component of the diluent for storage of semen has been investigated by several Russian workers. They report some success with several diluents at several storage temperatures. In one study, semen of Dagestan Mountain rams was diluted 1:10 in glucose-yolk-citrate, saturated with CO_2 and kept at 18° C (Dzabrailov and Sel'kin, 1962). Motility was reportedly maintained for 72 hours and 98.5 percent of over 9,000 ewes lambed from insemination with semen diluted in this manner, but storage time and number of services were not stated in the abstract.

One factor which has differed and which could be a key to success or failure is the rate at which the semen is cooled to the storage temperature. Often this rate has not been known precisely, but a slow rate of cooling has been shown to be important for deep-freezing in several studies including that by Jones (1969a). It is possible that cooling rate may be important for storage at or above

0°. Malikov (1960) using a storage temperature of 0° C reported a conception rate of 67 percent with semen cooled from 20 to 0° over 4 hours as compared to 54 percent with semen cooled by plunging the container directly into ice. Cold shock has been investigated in detail by Quinn and White (1967, 1968) and by Quinn *et al.* (1968b).

In early tests at West Virginia the N-J-1 and N-J-2 diluents formulated by Johnson and Norman (Norman, 1964) did not maintain fertility of ram sperm. One of two modified N-J-2 diluents developed by Johnson (unpublished) and buffered with Tes (N-tris-(hydroxymethyl) methyl-2 aminoethane sulfonic acid) showed some promise for storage at room temperature up to 50 hours when 2.0 ml. semen containing a total of 300 million sperm were deposited into the cervix of ewes at first estrus after progestogen treatment. Unfortunately, semen stored for 12 or 24 hours at room temperature in this diluent was ineffective in subsequent tests at volumes of 0.8, 1.2 and 1.6 ml. containing 75 or 150 million cells per ml.

Frozen semen

Long-term storage of ram semen in the frozen state is, of course, the eventual goal in studies of preservation of semen and is probably the ideal way to provide a readily accessible supply of high-quality semen from genetically superior sires. At the moment, however, this goal has not been realized. The most successful report cited by Emmens and Robinson (1962) was that by the Russian, Pokatilova, in 1960. He reported 46 percent of ewes lambing from insemination with semen which had been frozen to -21° C and stored at that temperature for five days. The semen was combined with a yolk-citrate-arabinose diluent, cooled from 20° to 0° in 30 to 60 minutes, held at 0° for four hours, rediluted 1:1 with the same diluent containing 15 percent glycerol (final concentration, 7.5 percent) and frozen slowly to -21° over at least 60 minutes.

Lapatko (1962) reported that 91 percent of 126 ejaculates diluted in glucose-yolk-citrate-glycerol showed less than 40 percent motility after storage for three to five months at -196°. He inseminated approximately 100 ewes in each of three field trials with this semen diluted 1:4. From 44 to 67 percent lambed from two doses of 0.2 ml. of this semen in the cervix at a 24-hour interval as compared to 40 to 78 percent of ewes bred with semen kept at 0°. At a dilution rate of 1:10, 41 percent of 106 ewes lambed. The diluent contained 100 ml. distilled water, 1.5 gm. glucose, 3.5 gm. sodium citrate and 15 ml. egg yolk. Fresh semen was diluted 1:1 with this mixture and cooled to 2 to 5° C in 1 hour. After cooling the semen was rediluted 1:1 with a medium containing 88 ml. of the same diluent and 12 ml. glycerol by layering in portions. The semen was frozen at rates of 0.5° per minute from +4 to -15° C, 2.0° per minute from -15 to -50°, 5 to 10° per minute from -50° to -75° and 100 to 200° per minute from -75 to 196°.

The use of hypertonic diluents may be helpful (Lopyrin and Loginova, 1958; Salamon and Lightfoot, 1969). Salamon and Lightfoot (1967) used semen diluted in hypertonic yolk-citrate-raffinose, frozen in pellets on dry ice, stored in liquid nitrogen for 4 to 12 weeks and thawed in 2.6 percent sodium citrate at 37° C. They compared cervical insemination with uterine and tubal insemination at laparotomy. In one experiment, the percentages of ova recovered which were fertilized were 25 with cervical, 88 with uterine, and 54 with tubal insemination. In a second experiment, 93 percent of 29 eggs were fertilized after uterine insemination but only 25 of 68 (34 percent) ewes did not return to estrus within 22 days. This high rate of loss of embryos suggests that the frozen sperm may have suffered genetic damage despite the fact that motility and fertilizing ability were retained. However, no data were given on embryo loss after uterine insemination with fresh semen, so infection and surgical trauma cannot be ruled out. Fraser (1968) has reported higher conception rates with intrauterine than with cervical insemination with semen frozen in pellets in an egg yolk-lactose-glycerol diluent.

In more recent work with ram semen, freezing in pellets and straws sometimes showed promise (Platov, 1965, 1968; Aamdal and Anderson, 1968; Sainsbury, 1968; Loginova and Zeltobrjuh, 1968). Pellets and ampules were superior to straws in studies by Salamon (1968) with motility after thawing being the criterion of recovery. Pellet freezing has been studied extensively by Salamon and Lightfoot (1967, 1969, 1970) and by Lightfoot and Salamon (1969a, b, 1970a, b). They reported that optimum tonicity varied with diluent composition, that sugars of higher molecular weight were superior and that best results were obtained by dilution with glycerol-containing diluent (3 to 4 percent) prior to cooling and a short storage period at 5° C prior to freezing. Pellet volume from 0.03 to 0.86 ml. had no effect. Better survival was obtained with thawing in glucose-citrate than in citrate solution. Semen was diluted no more than 1:3 before freezing and again during thawing. Best results (48 to 53 percent of ewes lambing) were obtained with 0.1 ml. of semen containing 1,500 to 1,600 million cells per ml. and two inseminations at a 12-hour interval.

Aamdal and Anderson (1968) reported 62 percent pregnancy in 26 ewes inseminated with semen frozen in straws. Colas and Brice (1970) in France also have used straws and with an egg yolk lactose diluent, they obtained 52 to 68 percent conception rates in PMS-treated ewes. In recent New Zealand work (Welch, Hulet, Shannon and Wilson, unpublished) semen has been diluted 3:1 (3 parts semen) with 70 percent egg yolk citrate, equilibrated in 8 percent glycerol and frozen in mini-straws in N^2 vapor. After thawing 16 seconds at 35° C it was inseminated immediately. Insemination into the cervix immediately at the onset of heat and 12 hours later at the second and third heats after progestin and PMS-treatment yielded 21 and 49 percent conception rates respectively. Insemination into the uterine horns in 38 ewes 12 hours after heat detection led

to 28 conceptions or 74 percent, but insemination into the oviducts gave only 35 percent conception as determined by pregnancy diagnosis. In this country, the only recent report is that of Dziuk *et al.* (1972) who obtained only 13 percent of 56 ewes lambing from AI with Finnish Landrace semen which had been diluted 1:3 in a Tes- and Tham-buffered egg yolk-fructose diluent containing 6.7 percent glycerol and frozen in pellets.

One must conclude that the lack of suitable methods for storing ram semen, particularly frozen storage, is the most important problem preventing introduction of AI on a practical basis in sheep. Solution of this problem will provide a strong impetus to the development of sheep AI programs. More work is needed to determine those combinations of dilution rates and freezing rates which will return maximum numbers of viable cells. Protective agents other than glycerol deserve investigation. Platov (1966a, b) reported favorable results with ethylene glycol and diethylene glycol in terms of motility after freezing, but Salamon (1968) obtained poorer results with ethylene glycol and dimethyl sulfoxide, alone or in combination with glycerol, than with 5 percent glycerol alone.

Recent studies on the metabolism of semen after freezing (O'Shea, 1969a, b) and on the effect of freezing on cell content of ATP (Platov, 1968) or loss of lactic dehydrogenase (Nath and Patt, 1970) provide important basic information. Such studies eventually may lead to knowledge which will enable easier freezing of ram sperm.

TIMING OF INSEMINATION

Artificial insemination should be timed so as to provide a maximum number of sperm which are capable of effecting fertilization at the site of fertilization when the ova reach this area shortly after ovulation. The site of fertilization has not been established precisely in the ewe, but is presumed to be in the upper half of the oviduct (Hancock, 1962). To achieve optimum fertility the time of ovulation, the number of sperm deposited, the time required for sperm and egg transport, the life span of sperm and eggs, and the possible need for capacitation of sperm must all be taken into consideration. Furthermore, season of the year and age of ewe are important time factors (Salamon and Robinson, 1962a). Single inseminations were less effective by 19 percentage points in the spring than in the fall. Conception rates to first service in virgin ewes were 8 to 15 percentage points lower than in older ewes. The latter effect may be due to age differences in the rate of egg transport (Edgar, 1962).

Both duration of overt behavioral estrus and the time of ovulation relative to the beginning or end of estrus have been shown to vary considerably within and between breeds, leading Robinson (1959) to state that "the only reliable conclusion to be drawn ... is that ovulation occurs at about the end of estrus." Parsons *et al.* (1967) cited four early papers which indicated that ovulation generally occurs a minimum of 24 hours after the onset of heat. Their own work

supported this conclusion. The peak of LH release at natural estrus occurs at or within 16 hours after the onset of standing heat (Geschwind, 1972). The interval from LH release to ovulation lies between 21 and 26 hours (Geschwind, 1972).

One interesting problem with respect to AI is the effect of association or lack of association with rams on the time of ovulation after the beginning of estrus. Early work by McKenzie and Terrill (1937) indicated that coitus shortened estrus. Russian workers (Zeltobrjuh and Rak, 1964) reported that multiple matings at 2-hour intervals shortened both the duration of estrus and the interval to ovulation as compared to ewes separated from the rams for 24 hours after first mating. Continuous association with rams also shortened estrus as compared to teasing at 4-hour intervals in Merino ewes in South Africa (Parsons and Hunter, 1967). Ovulation occurred 5 hours later in the constantly teased ewes, however (Parsons *et al.,* 1967). Parsons and Hunter (1967) found that synchronization of estrus with progesterone had no effect on length of heat or its reduction by the ram, but they did not determine ovulation time in the ewes treated with progesterone. Lewis, Bolt, and Inskeep (unpublished) have found that progestin treatment may alter the time of LH release with respect to onset of estrus (Figure 2).

Robinson (1961) reported that ewes treated with injections of progesterone or progesterone and PMS usually ovulated 12 to 24 hours after heat was detected by twice daily observations. However, the period during which ovulation occurred was at least 36 hours in length. Robinson and Moore (1967) obtained conception rates of 49 and 56 percent by AI at 48 and 52 hours after heat was detected. Robinson and Smith (1967) found that ovulation usually occurred 48 to 66 hours after removal of sponges containing 10 to 20 mg. of SC-9880 but 66 to 84 hours after removal of sponges containing 40 mg. of this compound.

Considerable controversy has developed in regard to the length of time required for transport of spermatozoa from the vagina to the upper half of the oviduct in both sheep and cattle. About half of the studies have found sperm in the tube within a few minutes, whereas the remainder have found sperm only after several hours (Hancock, 1962). Mattner and Braden (1963) reported that the rapid phase of sperm transport (during the first few minutes after coitus) was interfered with by the stress resulting from rough handling. In later work from France (Thibault and Wintenberger-Torres, 1967) sperm were seldom found in the oviducts by two hours after mating, but handling stress did produce a reduction in transport of sperm to the upper uterine horn.

Restall (1961) reported that conception rate to AI is increased by 6 to 10 percentage points by placing ewes with teaser (vasectomized) rams for several hours following insemination. Hawk (1972) found no effect of teaser rams after AI on sperm transport or conception rate. The French study cited above also reported that injection of oxytocin increased sperm transport in mated ewes not

subject to the handling stress. However, the injection of oxytocin immediately after artificial insemination with fresh, undiluted semen did not increase conception rate in Corriedale ewes (Jones *et al.*, 1969). Jones (1968) found no value of oxytocin injection following insemination with diluted semen.

It was pointed out earlier that both Australian workers (Moore *et al.*, 1967; Quinlivan and Robinson, 1967) and American workers (Hawk and Conley, 1971,

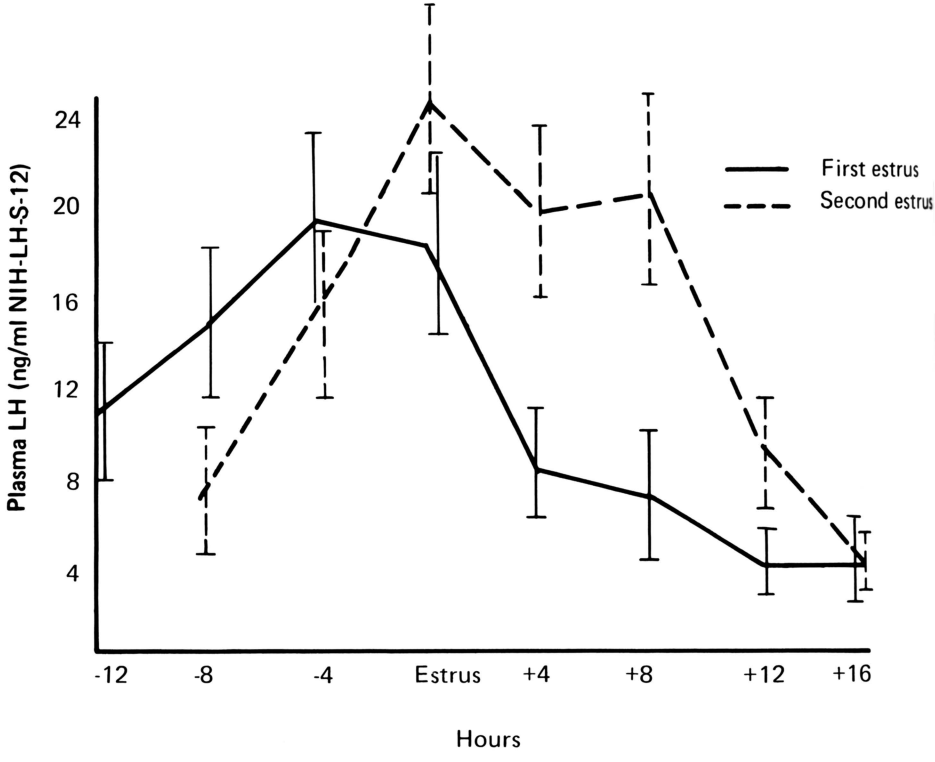


FIGURE 2

Mean LH release in reference to onset of estrus for first and second post-treatment estrous periods.

1972) have demonstrated that sperm transport is considerably reduced at synchronized estrus. Thus a lesser degree of success can be expected when diluted semen and smaller numbers of sperm are used for AI in animals in which estrus and ovulation has been controlled. This is particularly true with treatment in the spring (Robinson, 1971; Inskeep, unpublished observations).

Shelton and Moore (1967) found that the rate of recovery of ova from the oviduct at a standard time was reduced by treatment with some progestogens. They also found that fertilized ova recovered from progestogen-treated ewes were at an earlier stage of development if the animals had been inseminated artificially than if they had been mated. This relationship was not seen in control ewes. Natural service occurred as much as 24 hours prior to AI in both the treated and control ewes so that alterations in ovum transport, sperm transport and ovulation time are all possible explanations for this effect.

According to Hancock (1962) the estimates of the functional life span of ram sperm in the reproductive tract of the ewe have varied from 22 to 48 hours and breed differences are to be expected. Several studies have shown that survival of diluted and stored sperm, particularly if frozen, may be reduced considerably (Loginova and Zeltobrjuh, 1968; Mattner *et al.*, 1969). Eggs in sheep are estimated to be viable for only 15 to 24 hours (Hancock, 1962), but Emmens and Robinson (1962) have stated that they may be readily fertilizable for only 8 to 10 hours. Fluids from different areas of the female tract vary in their effects on survival of ram sperm *in vitro* (Restall, 1969). Oviduct fluid, which did not maintain sperm well in Restall's study, stimulated respiration of ram sperm in work by Black *et al.* (1968).

Despite the fact that Chang (1967) lists the sheep as an animal in which spermatozoa must undergo a capacitation in the female tract in order to be capable of fertilization of eggs, the evidence that this is the case is insufficient. A search of the literature revealed only two papers on the subject. The first was a report by Dauzier and Thibault (1959) on their attempt to fertilize sheep eggs in test tubes. With fresh semen, none of 41 eggs showed any signs of fertilization. With sperm flushed from the female tract 12 to 24 hours after mating, four of 78 eggs were judged to have been fertilized. The second paper was that of Mattner (1963) in which he reported that he placed fresh semen into the oviduct of sheep that had just ovulated. He found that the time required for penetration of the zone pellucida was approximately 1.5 hours and the sperm head was not within the vitellus for 2.5 to 3 hours. He did not use semen which had been in the female tract for comparison with fresh semen, but concluded that capacitation of ram sperm in the oviduct required about 1.5 hours.

The need for accurate timing was emphasized by Jones *et al.* (1969) who found a relationship of conception rate to both stage of estrus and mucus score as had been reported earlier by numerous workers.

Practical considerations

Heat detection usually is accomplished by aproned or vasectomized rams. Russian workers (Milovanov *et al.*, 1960) turn these rams in with the ewes in shifts beginning at daybreak and draft off the ewes for insemination as they are detected in heat. Salamon and Morrant (1963) found more ewes in heat when painted teaser rams were run with the ewes constantly, than when they were introduced only for a short period. Removal of the ewes in heat at least twice daily will increase the efficiency of heat detection and reduce the required number of teaser rams.

Some workers have concluded that ewes should be inseminated only once per heat period since the increases in conception and twinning rates with additional inseminations were rather low (Salamon and Robinson, 1962a; Dunlop and Tallis, 1964). Using frozen semen, Lopatko (1962) found an improvement in conception rate of 8 percentage points with two inseminations per heat period (50 vs. 42 percent). It appears that the standard procedure in Australia is to check heat once daily, early in the morning, and inseminate during the next few hours. Similarly, Milovanov *et al.* (1960) recommended insemination as soon as the ewe is found in heat. They further proposed reinsemination 24 to 30 hours later in cases of prolonged heat. Terrill (1960) recommended twice daily heat checks and breeding each time the ewe is in heat. However, he suggested breeding 12 to 14 hours after the ewe is first observed in heat if a single insemination is used. Subsequent studies have supported his conclusion for ewes at normal estrus or at second estrus after progestin treatment. Conception rate at 12 hours after onset of estrus was 18 percentage points higher with fresh, undiluted semen and 12 percentage points higher with diluted, stored semen than when the ewes were bred as soon as estrus was detected in studies made by Stevens (1970) at West Virginia. Amir and Schindler (1972) in Israel obtained maximum conception rates to fresh semen in Awassi ewes from 4 to 36 hours after onset of estrus as determined by heat checks every four hours. mating in ewes treated with progestin and HCG is about 12 hours before expected ovulation. However, Dziuk *et al.* (1972) inseminated at this time with fresh, undiluted semen and obtained conception rates of 21 and 20 percentage points lower than with natural mating during fall and spring, respectively. Robinson and Moore (1967) obtained equal conception rates with timed insemination or insemination after detection of heat at the first estrus after progestin treatment, but rates were low in both cases, averaging around 50 percent. With two inseminations at 36 and 48 hours after removal of Cronolone-sponges, Peters, Kauf and Inskeep (unpublished) obtained 60 percent conception in one flock of 29 ewes compared to 62 to 77 percent in three other flocks in which ewes were inseminated only once, 12 or 24 hours after onset of second post treatment heat. English workers, McClelland and Quirke (1971, cited by Gordon, 1972) obtained 87 percent conception by AI at 48 and 58

hours after Cronolone withdrawal in 30 Finn x Dorset ewes, compared to 94 percent with hand mating at the same times in 31 ewes.

SITE OF SEMEN DEPOSITION

The usual site of semen deposition in artificial insemination has been just within the cervix, usually to a depth of not more than one-quarter inch. Efficiency in finding the opening of the cervix will vary with the equipment used, the age of the ewe, and the experience of the operator. The cervical opening has been reported to be easier to find in the virgin ewes. The form of the os cervix varies considerably from animal to animal (Figure 3). Most studies have shown cervical insemination to be considerably superior to vaginal insemination although Koger (1951) found no difference and Tjupic (1959) was quite successful with vaginal insemination.

Little attention has been given to the possibility of uterine insemination in field trials. The cervical canal is small and tortuous and varies considerably in length (Figure 3). With a conscious effort, however, it is possible to reach the uterus in some ewes with a tapered inseminating tube or a 12-guage metal inseminating tube 18 inches in length. This may be advantageous in out-of-season breeding or at synchronized estrus when sperm transport can be expected to be impaired. The improved fertilization rate with uterine insemination of frozen semen was mentioned earlier. For these reasons, further studies of uterine insemination and of equipment for this purpose deserve attention.

EQUIPMENT FOR INSEMINATION

The ewe is too small to be inseminated by manipulation of the cervix through the rectal wall as is done for a cow. Rather, a means to see the cervix is necessary. The equipment for artificial insemination has been discussed in some detail by Emmens and Robinson (1962). Several approaches have been made to restraining the ewe so that the reproductive tract is at eye level of the inseminator. These include (1) placing the inseminator in a pit behind the ewe, which is either held by an assistant or fastened in a head stall, (2) placing the ewe on a so-called rail crate developed at the Trangie Experiment Station in New South Wales, the rear of which is then elevated so that the inseminator looks down into the reproductive tract, (3) use of a simple crate on legs into which the ewe is lifted by assistants and which can be transported easily (Figure 4), and (4) use of a rotating platform with three stalls, one of which is being loaded and another of which is being unloaded while the third contains the ewe being inseminated (Figure 5). The latter system can be used to inseminate 300 or more

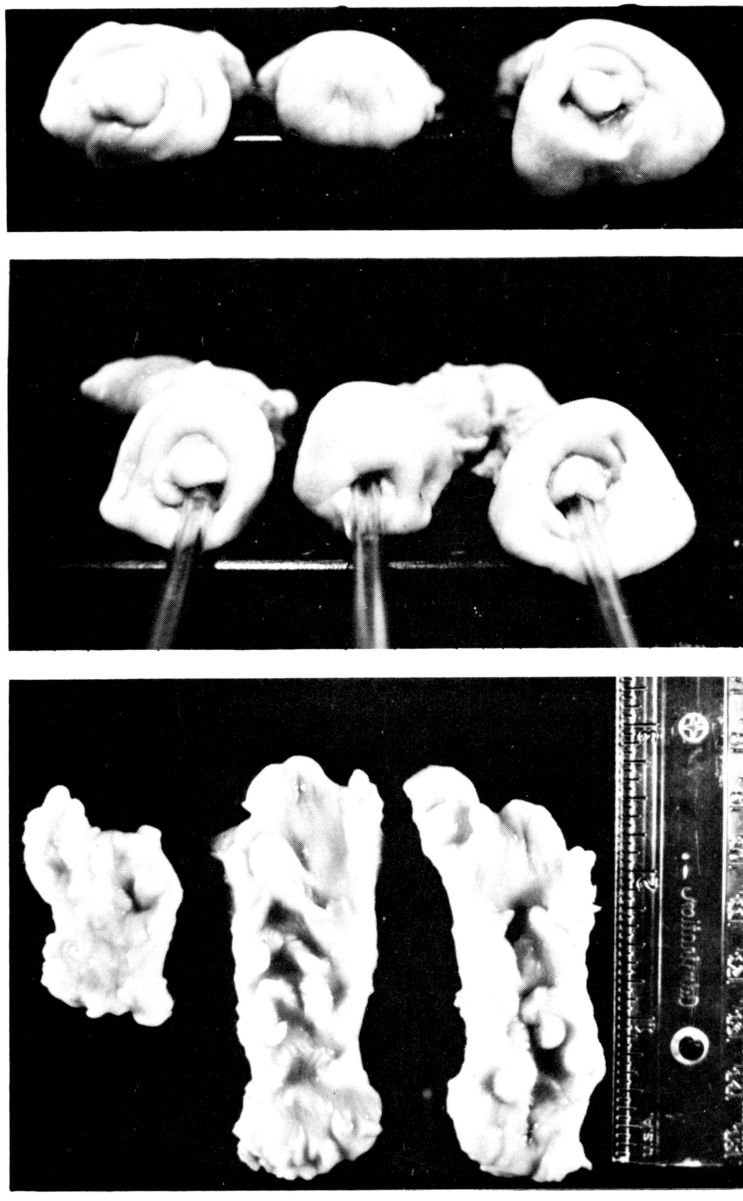


FIGURE 3

Morphology of uterine cervix in three whitefaced ewes. Top—os cervix as it would be seen using a vaginal speculum. Middle—os cervix with tapered inseminating tube inserted deep into cervix. Bottom—cervical canal as seen when opened by a longitudinal incision (os cervix is at top of photograph).

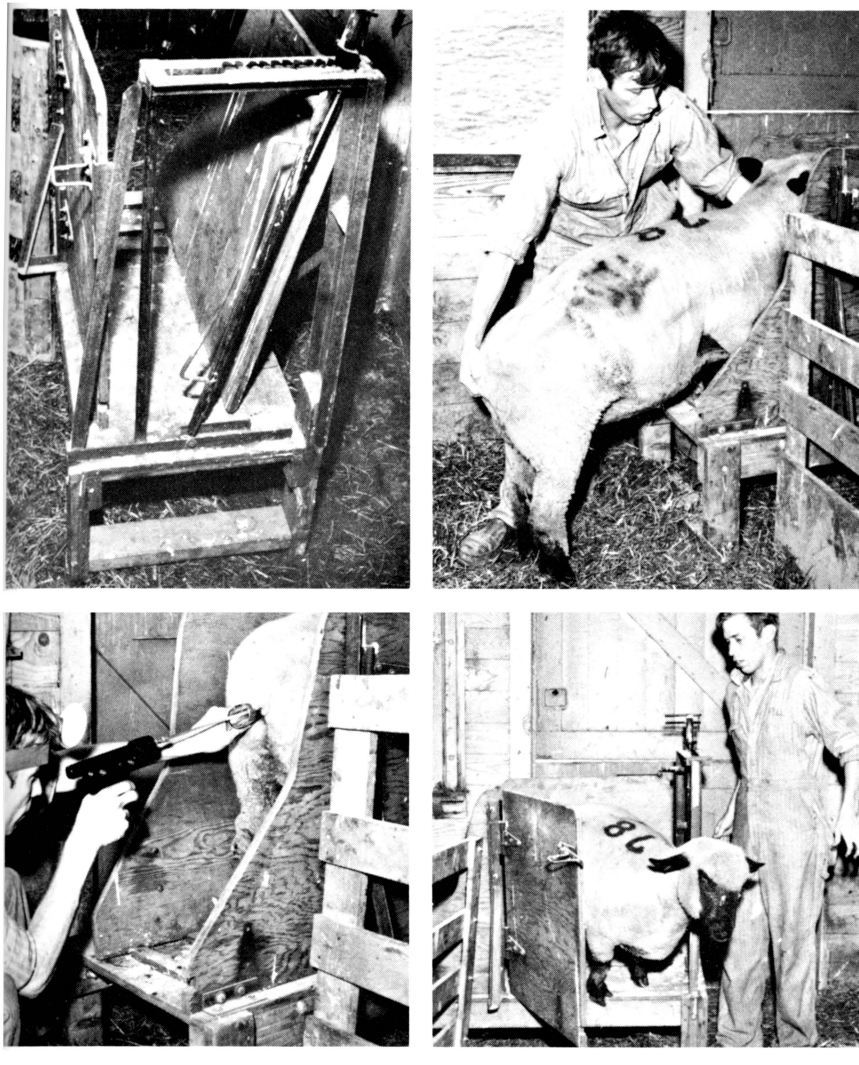


FIGURE 4

Insemination crate used at West Virginia station. Upper left—front view of crate showing spring-loaded head catch and side door. Upper right—loading ewe into crate. Lower left—inseminating ewe with multiple-dose injection gun. Lower right—the ewe is released into another pen by the side door.

FIGURE 5

Three tilting squeeze chutes mounted on a rotating platform in association with a New Zealand style sheep handling corral can be used for artificial insemination, pregnancy diagnosis and foot trimming.

ewes per day with a four-man crew. For large-scale operations, the rotating platform in connection with an elevated loading pen and an unloading ramp appears to be the most efficient of these approaches.

A portable squeeze stall used at the West Virginia Station for small flocks is illustrated in Figure 4. It includes a spring-loaded head catch mechanism. One side of the stall is attached by a spring to the movable side of the stanchion. The front legs of the ewe are placed into the back of the stall and she is then pushed forward until her head can be fastened. The other side of the stall is a door through which the ewe exits when she is released. The stall is usually set into the side of a temporary catch pen made of hurdles at an angle so that the inseminated ewe is released outside the catch pen. The inseminator kneels or sits on a low stool.

The duckbill speculum is a satisfactory means to open the vagina to locate the cervix. Depending on location of the breeding pen and time of day, either a standard miner's headlamp or the sun is used as a source of light. Inseminating syringes vary from simple tubes with syringes attached to semi-automatic units capable of holding 10 to 40 doses of semen (Emmens and Robinson, 1962).

CONCLUSIONS

There are several problems which must be solved before artificial insemination becomes a practical reality in sheep in the United States. Two that stand out are the lack of satisfactory methods for frozen storage of ram semen and the lowered fertility to AI at controlled estrus. It appears that greater numbers of sperm will be required in sheep than in cattle. The development of techniques for uterine insemination may help to reduce this requirement and should be investigated.

For the present, an AI program in sheep probably should include (a) twice daily heat checks, (b) insemination into the cervix 12 to 24 hours after the ewe is found in heat, and (c) use of fresh semen, diluted no more than 1:2. Such a program can be carried out successfully at the second estrus after ovulation control by progestogen treatment with conception rates in excess of 60 percent.

The genetic gain that may be realized from the use of superior sires in AI, particularly in the areas of prolificacy and growth rate, should be sufficient to justify considerable effort towards the solution of those problems which limit the practical use of AI today.

ACKNOWLEDGMENTS

The financial support of G. D. Searle and Co. and the assistance of Mr. Charles E. Cooke in searching the literature prior to preparation of this review are gratefully acknowledged.

LITERATURE CITED

Aamdal, J. and K. Andersen. 1968. Freezing of ram semen in straws. VIth Int. Congr. Animal Reprod. and A.I. 2:977.

Allison, A. J. and T. J. Robinson. 1970. The effect of dose level on intravaginal progestagen on sperm transport, fertilization, and lambing in the cyclic Merino ewe. *J. Reprod. Fert.* 22:515.

Almquist, J. O. 1959. Insemination techniques. In *Reproduction in Domestic Animals*, H. H. Cole and P. T. Cupps, Eds.; Academic Press, New York, Vol. II, p. 135.

Amir, D. and H. Schindler, 1972. The conception rate of ewes after artificial insemination at different times during oestrus. *J. Reprod. Fert.* 28:261.

Black, D. L., L. V. Crowley, R. T. Duby and C. H. Spilman. 1968. Oviduct secretion in the ewe and the effect of oviduct fluid on oxygen uptake by ram spermatozoa *in vitro*. *J. Reprod. Fert.* 15:127.

Chang, M. C. 1967. Summation of workshop. *J. Reprod. Fert.*, Suppl. 2:145.

Colas, G. and G. Brice, 1970. Fertility of flurogestone acetate-treated ewes inseminated with frozen sperm: preliminary results. *Ann. Zootech.* 19(3)353.

Dauzier, L. and C. Thibault. 1959. Donnees nouvelles sur la fecondation *in vitro* de l'oeuf la lapin et de la brebis. C. R. Acad. Sci. Paris 248:2655.

Dauzier, L., C. Thibault and S. Wintenberger. 1954. Conservation du sperme de belier apres dilution et maintien de son pouvoir fecondant. *Ann. Endocr.* 15:341.

Dunlop, A. A. and G. M. Tallis. 1964. The effects on length of estrous and number of inseminations on the fertility and twinning rate of the Merino ewe. *Aust. J. Agr. Res.* 15:282.

Dziuk, P., J. M. Lewis, E. F. Graham and R. H. Moyer. 1972. Comparison between nature service and artificial insemination with fresh or frozen sperm at an appointed time in the ewe. *J. Animal Sci.* 35:572.

Dzabrailov, D. M. and I. I. Sel'kin. 1962. Gaseation of ram semen diluent. Ovcevodstov 8 (9):29. (*Animal Breed. Abstr.* 31:80, 1963).

Edgar, D. G. 1962. Studies on infertility in ewes. *J. Reprod. Fert.* 3:50.

Emmens, C. W. and T. J. Robinson. 1962. Artificial insemination in the sheep. In *The Semen of Animals and Artificial Insemination,* J. P. Maule, Ed; Commonwealth Agricultural Bureau, Edinburg, p. 205.

Entwistle, K. W. and I. C. A. Martin. 1972. Effects of the number of spermatozoa and of volume of diltued semen on fertility in the ewe. *Aust. J. Agr. Res.* 23:467.

First, N. L., A. Sevinge and H. A. Henneman. 1961. Fertility of frozen and unfrozen ram semen. *J. Animal Sci.* 20:79.

Fraser, A. F. 1968. Progress in the artificial insemination of sheep with frozen semen. VIth Int. Congr. Animal Reprod. and A. I. 2:1033.

Geschwind, I. I. 1972. Dynamics of pituitary gonadotropin secretion. *J. Animal Sci.* 34:Suppl. I:19.

Gordon, I. 1972. Control of reproduction in sheep: towards programmed lamb production. *J. Dept. Agr. and Fish.* (Dublin) 68:1.

Hancock, J. L. 1962. Fertilization in farm animals. *Animal Breed. Abstr.* 30:285.

Hawk, H. W. 1972. Progestagen induced sperm breakage in the sheep vagina. *J. Animal Sci.* 34:795.

Hawk, H. W. and H. H. Conley. Loss of spermatozoa from the reproductive tract of the ewe and intensification of sperm breakage by progestagen. *J. Reprod. Fert.* 27:339.

Hawk, H. W. and H. H. Conley, 1972. Investigation of sperm transport failures in ewes administered synthetic progestagen. *J. Animal Sci.* 34:609.

Johnson, C. E., R. L. Reynolds, C. Norman, H. E. Kidder and D. J. Horvath. 1963. Artificial insemination of swine with sperm stored at room temperature. *A. I. Digest* 16 (6):8.

Jones, R. C. 1968. The fertility of ewes injected with synthetic oxytocin following artificial insemination. *Aust. J. Exp. Agr. Animal Husb.* 8:13.

Jones, R. C. 1969a. Influence of diluents and processing times after ejaculation on the survival of deep frozen ram spermatozoa. *Aust. J. Biol. Sci.* 22:995.

Jones, R. C. 1969b. Studies of the suitability of preparations of ewe and cow milk for storing ram spermatozoa. *Aust. J. Biol. Sci.* 22:983.

Jones, R. C., I. C. A. Martin and K. R. Lapwood. 1969. Studies of the artificial insemination of sheep: The effects on fertility of diluting ram semen. Stage of oestrus of the ewe at insemination and injection of synthetic oxytocin. *Aust. J. Agr. Res.* 20:141.

Koger, M. 1951. Storage, dilution and use of ram semen in artificial breeding of sheep. New Mex. Agr. Exp. Sta. Bull. 366.

Lapwood, K. R., I. C. A. Martin and K. W. Entwistle. 1972. The fertility of Merino ewes artificially inseminated with semen diluted in solutions based on skim milk, glucose or ribose. *Aust. J. Agr. Res.* 23:457.

Lightfoot, R. J. and S. Salamon. 1969a. Freezing ram spermatozoa by the pellet method. *Aust. J. Biol. Sci.* 22:1547.

Lightfoot, R. J. and S. Salamon. 1969b. Freezing ram spermatozoa by the pellet method. *Aust. J. Biol. Sci.* 22:1561.

Lightfoot, R. J. and S. Salamon. 1970a. Fertility of ram spermatozoa frozen by the pellet method. *J. Reprod. Fert.* 22:385.

Lightfoot, R. J. and S. Salamon. 1970b. Fertility of ram spermatozoa frozen by the pellet method. II. The effects of method of insemination on fertilization and embryonic mortality. *J. Reprod. Fert.* 22:399.

Loginova, N. V. and N. A. Zeltobrjuh. 1968. Experimental test of different methods of ram sperm freezing. VIth Int. Congr. Animal Reprod. and A. I. 2:1077.

Lopatko, M. I. 1962. Method of freezing ram semen to -183° or -196°. *Zivotnovodstvo* 24 (10):86.

Lopyrin, A. I. and N. V. Loginova. 1958. Methods of freezing ram semen. *Ovcevodstvo* 4 (8):31.

Lunca, N. 1964. Artificial insemination in sheep and goats on the international plane. Vth Int. Congr. Animal Reprod. and A. I. 4:118.

Malikov, D. I. 1960. Slow cooling of semen increases its activity. *Ovcevodstvo* 6 (9):35. (*Animal Breed. Abstr.* 29:72, 1961).

Malikov, D. I. 1963. The preservation of ram semen at temperatures above freezing point. *Ovcevodstvo* 9 (9):20. (*Animal Breed. Abstr.* 32:189, 1964).

Mann, T. 1964. *The Biochemistry of Semen,* Metheun and Co., London.

Martin, I. C. A. 1968. Milk and synthetic diluents for ram semen. VIth Int. Congr. Animal Reprod. and A. I. 2:1619.

Mattner, P. E. 1963. Capacitation of ram spermatozoa and penetration of the ovine egg. *Nature* 199:772.

Mattner, P. E. and A. W. H. Braden. 1963. Spermatozoa in the genital tract of the ewe. *Aust. J. Biol. Sci.* 16:473.

Mattner, P. E. and J. K. Voglmayr. 1962. A comparison of ram semen collected by the artificial vagina and by electroejaculation. *Aust. J. Exp. Agr. Animal Husb.* 2:78.

Mattner, P. E., K. W. Entwistle and I. C. A. Martin. 1969. Passage, survival, and fertility of deep frozen ram semen in the genital tract of the ewe. *Aust. J. Biol. Sci.* 22:181.

McKenzie, F. F. and C. E. Terrill. 1937. Estrus, ovulation and related phenomena in the ewe. Missouri Agr. Exp. Sta. Res. Bull. 264.

Milovanov, V. K., G. D. Trubkin, N. S. Chubenko, I. V. Isvetkov, A. K. Erzin and A. B. Meschankin. 1960. *Artificial Insemination of Livestock in the USSR,* Ministry of Agric. RSFSR, Moscow. Translated and published for USDA and NSF by Israel Program for Scientific Translations, 1964.

Moore. N. W., T. D. Quinlivan, T. J. Robinson and J. F. Smith. 1967. The fertilization of ova following natural mating or artificial insemination after withdrawal of SC-9880-impregnated intravaginal sponges from cyclic Merino ewes. In T. J. Robinson, *The Control of the Ovarian Cycle in the Sheep,* Sydney University Press, p. 169.

Nath, J. and J. A. Patt, Jr. 1970. Biochemical changes associated with freezing in ram semen. *Cryobiology* 6:522.

Norman, C. 1964. Further studies on the preservation of mammalian sperm at variable temperatures. Vth Int. Cong. Animal Reprod. and A. I. 4:269.

O'Shea, T. 1969a. Metabolism of semen after freezing: The effects of washing, skim milk solids, and potassium and magnesium ions on the metabolism of thawed ram and bull spermatozoa. *Aust. J. Biol. Sci.* 22:721.

O'Shea, T. 1969b. Metabolism of semen after freezing: The aerobic metabolism of ram spermatozoa before and after storage at -79°C. *Aust. J. Biol. Sci.* 22:733.

Parsons, S. D. and G. L. Hunter. 1967. Effect of the ram on duration of oestrus in the ewe. *J. Reprod. Fert.* 14:61.

Parsons, S. D., G. L. Hunter and A. A. Rayner. 1967. Use of probit analyses in a study of the effect of the ram on the time of ovulation in the ewe. *J. Reprod. Fert.* 14:71.

Platov, E. M. 1965. The freezing of ram semen in a lactose-yolk-ethylineglycol diluent. *Ovcevodstvo* 11 (9):11. (*Animal Breed. Abstr.* 34:76, 1966).

Platov, E. M. 1966a. The use of diethylene glycol as a protectant for bull and ram semen during freezing. *Khim. Khoz.* 6:42-44. (*Animal Breed. Abstr.* 35:191, 1967).

Platov, E. M. 1966b. The problem of freezing ram semen. Tryd. Inst. Zhivot. 29:355. (*Animal Breed. Abstr.* 35:634, 1967).

Platov, E. M. 1968. The effect of freezing on the content of adenosine polyphosphates in semen. *Ovcevodstvo* 14 (8):14. (*Animal Breed. Abstr.* 37:16, 1969).

Pokatilova, G. A. 1960. Storing ram semen at -21°C. *Ovcevodstvo* 6 (1):23. (*Animal Breed. Abstr.* 28:158, 1960).

Quinlivan, T. D. and T. J. Robinson. 1967. The number of spermatozoa in the Fallopian tubes of ewes at intervals after artificial insemination following withdrawal of SC-9880 impregnated intravaginal sponges. In T. J. Robinson, *The Control of the Ovarian Cycle in the Sheep,* Sydney University Press, p. 177.

Quinlivan, T. D. and T. J. Robinson. 1969. Numbers of spermatozoa in the genital tract after artificial insemination of progestagen-treated ewes. *J. Reprod. Fert.* 19:73.

Quinlivan, T. D. 1970. Relationship between numbers of spermatozoa inseminated and

fertilization rate of ova in ewes treated with fluoro-progestagen intravaginal sponges in summer and autumn. *J. Reprod. Fert.* 23:87.

Quinn, P. J. and I. G. White. 1966. Variation in semen cations and its relation to semen quality and methods of collection. *Fert. Steril.* 17:815.

Quinn, P. J. and I. G. White. 1967. Phospholipid and cholesterol content of epididymal and ejaculated ram spermatozoa and seminal plasma in relation to cold shock. *Aust. J. Biol. Sci.* 20:1205.

Quinn, P. J. and I. G. White. 1968. The effect of pH, cations, and protective agents on the susceptibility of ram spermatozoa to cold shock. *Exp. Cell Res.* 49:31.

Quinn, P. J., S. Salamon and I. G. White. 1968a. The effect of cold shock and deep freezing on ram spermatozoa collected by electrical ejaculation and by an artificial vagina. *Aust. J. Agr. Sci.* 19:119.

Quinn, P. J., S. Salamon and I. G. White. 1968b. Effect of osmotic pressure and temperature gradients on cold shock in ram and bull spermatozoa. *Aust. J. Biol. Sci.* 21:133.

Restall, B. J. 1961. Artificial insemination of sheep. *Aust. Vet. J.* 37:70.

Restall, F. B. 1969. The influence of the fluids from the genital tract of the ewe on the survival of ram spermatozoa. *Aust. J. Biol. Sci.* 22:1517.

Roberts, E. M. and P. Houlahan. 1961. The comparison of the fertility of stored and fresh sheep semen. *Aust. J. Exp. Agr. Animal Husb.* 1:156.

Robinson, T. J. 1956. The artificial insemination of the Merino sheep following the synchronization of oestrus and ovulation by progresterone injected alone and with pregnant mare serum gonadotrophin (PMS). *Aust. J. Agr. Res.* 7:194.

Robinson, T. J. 1958. Studies in controlled artificial insemination of Merino sheep. *Aust. J. Agr. Res.* 9:693.

Robinson, T. J. 1959. The estrous cycle of the ewe and doe. In *Reproduction in Domestic Animals,* H. H. Cole and P. T. Cupps, Eds.; Academic Press, New York Vol. I, p. 291.

Robinson, T. J 1961. The time of ovulation and efficiency of fertilization following progesterone and pregnant mare serum treatment in the cyclic ewe. *J. Agr. Sci.* 57:129.

Robinson, T. J. 1967. Editor, *The Control of the Ovarian Cycle in the Sheep,* Sydney University Press, Sydney.

Robinson, T. J. 1970. Fertility following synchronization of oestrus in the sheep with intravaginal sponges. *Aust. J. Agr. Res.* 21:783.

Robinson, T. J. 1971. The seasonal nature of reproductive phenomena in sheep. II. Variation in fertility following synchronization of oestrus. *J. Reprod. Fert.* 24:19.

Robinson, T. J. and N. W. Moore. 1967. The evaluation of progesterone and SC-9880-impregnated intravaginal sponges for the synchronization of oestrus for large scale artificial insemination of Merino ewes in spring. In T. J. Robinson, *The Control of the Ovarian Cycle in the Sheep,* Sydney University Press, p. 116.

Robinson, T. J. and J. F. Smith. 1967. The time of ovulation after withdrawal of SC-9880-impregnated intravaginal sponges from cyclic Merino ewes. In T. J. Robinson, *The Control of the Ovarian Cycle in the Sheep,* Sydney University Press, p. 158.

Rowson, L. E. A. 1959. Techniques of collection, dilution and storage of semen. In *Reproduction in Domestic Animals,* H. H. Cole and P. T . Cupps, Eds.; Academic Press, New York, Vol. II, p. 95.

Sainsbury, R. W. 1968. Freezing of ram semen in Cassou straws. VIth Int. Congr. Animal Reprod. and A. I. 2:1151.

Salamon, S. 1962. Studies on the artificial insemination of Merino sheep. *Aust. J. Agr. Res.* 13:1137.

Salamon, S. 1964. The effect of frequent ejaculation in the ram on some semen characteristics. *Aust. J. Agr. Res.* 15:950.

Salamon, S. 1968. Deep freezing of ram semen: Recovery of spermatozoa after pelleting and comparison with other methods of freezing. *Aust. J. Biol. Sci.* 21:351.

Salamon, S. and R. J. Lightfoot. 1967. Fertilization and embryonic loss in sheep after insemination with deep frozen semen. *Nature* 216:194.

Salamon, S. and R. J. Lightfoot. 1969. Freezing ram spermatozoa by the pellet method. *Aust. J. Biol. Sci.* 22:1527.

Salamon, S. and R. J. Lightfoot, 1970. Fertility of ram spermatozoa frozen by the pellet method. III. The effects of insemination techniques, oxytocin and relaxin on lambing. *J. Reprod. Fert.* 22:409.

Salamon, S. and A. J. Morrant. 1963. A comparison of two methods of artificial breeding in sheep. *Aust. J. Exp. Agr. Animal Husb.* 3:72.

Salamon, S. and T. J. Robinson. 1962a. Studies on the artificial insemination of Merino sheep. *Aust. J. Agr. Res.* 13:52.

Salamon, S. and T. J Robinson. 1962b. Studies on the artificial insemination of Merino sheep. *Aust. J. Agr. Res.* 13:271.

Schindler, H., E. Eyal and D. Amir. 1972. Fourth Annual Report. P. L. 480 Project, Israeli ARO, Volcani.

Shelton, J N. and N. W. Moore. 1967. The evaluation of alternative methods of administration of progestagens in the entire cyclic ewe. In T. J. Robinson, *The Control of the Ovarian Cycle in the Sheep,* Sydney University Press, p. 59.

Stevens, J. T. 1970. Thiamine propyl disulfide and β-amylase in ram semen diluents. M. S. Thesis, West Virginia University.

Terrill, C. E. 1960. Sheep and goats. In E. J. Perry, *The Artificial Insemination of Farm Animals,* Rutgers University Press, New Brunswick, p. 178.

Thibault, C. and S. Wintenberger-Torres. 1967. Oxytocin and sperm transport in the ewe. *Int. J. Fert.* 12:410.

Tjupic, M. M. 1959. Artificial insemination of sheep by vaginal method. *Ovcevodstvo* 5 (10):24. (*Animal Breed. Abstr.* 28:56, 1960).

Wiggan, L. S. and J. B. K. Clark. 1967. Observation on the fertility and quality of diluted and stored ram semen used in artificial insemination. *Brit Vet. J.* 123:447.

Zeltobrjuh, N. A. and L. P. Rak. 1964. Biological stimulation of the reproductive functions of the ewe. *Ovcevodstvo* 10 (8):8. (*Animal Breed. Abstr.* 33:94, 1965).

PART 2

EFFECT OF β-AMYLASE AND TIME OF INSEMINATION ON CONCEPTION RATES OF EWES INSEMINATED ARTIFICIALLY

J. T. Stevens and E. K. Inskeep

Austin (1951) and Chang (1951) observed that rabbit spermatozoa, although present at the site of fertilization in a very short time after insemination, required about six hours residency in the female reproductive tract before penetration of the ovum (this interval was designated capacitation time). Kirton and Hafs (1965) eliminated the need for a capacitation period in the rabbit by prior addition of β-amylase to sperm. In later studies (Kirton *et al.*, 1968), α- and β-amylases significantly increased fertility in cows bred artificially. Capacitation may be necessary prior to fertilization in the ewe (Dauzier and Thibault, 1959; Mattner, 1963). Two experiments were designed to determine the effects of adding β-amylase to extended ram semen and of time of insemination in relation to time of detected estrus on conception rates of ewes.

EXPERIMENTAL PROCEDURES

The two experiments involved 433 ewes of mixed breeds and ages. All ewes were treated for fourteen days with intravaginal pessaries (sponges) containing flurogestone acetate (Cronolone) in order to control ovulation. Semen was collected with an artificial vagina from five mature Hampshire rams. Semen diluted for artificial insemination (AI) was cooled over a three-hour period and stored for 12 hours at 5° C. Rams known to produce motile spermatozoa in high concentration were used for natural mating (NM). Results were analyzed by the contingency chi-square test (Fryer, 1966).

Experiment 1

At the time of pessary removal in November, 1968, 78 ewes were selected at random for NM to 8 rams and 160 ewes were allotted to a 2 X 2 factorial design for AI at 48 hours after pessary removal. Estrus was expected at 30 to 48 hours on the basis of prior studies (Inskeep, unpublished data). Spermatozoa were diluted 1:2 in either a TES (N-tris-[hydroxymethyl] methyl-2-amino

ethane sulfonic acid)-buffered egg yolk diluent (TEY) (C. E. Johnson, unpublished) or a standard egg yolk glucose citrate diluent (EYCG) (Ozin, 1956). Diluent compositions are presented in Table 1. Semen suspended in each diluent was divided into two fractions; β-amylase (Nutritional Biochemical Company) 2 mcg/ml, was added to one portion and the other served as a control.

The freshly diluted semen was placed into glass cartridges for storage and subsequent use in a modified "MeterMatic" (Squibb) multiple dose injector. Ewes inseminated artificially received 1.0 ml. of semen diluted to a final concentration of 123 X 10^6 cells/ml. in TEY or EYGC diluents, with or without β-amylase, into the cervical canal just anterior to the os cervix. When this was not possible, the insemination was recorded as "vaginal."

TABLE 1

Composition of the TES-Buffered[a] and Egg Yolk-Citrate Diluents

Constituent	Diluents	
	TEY gm/liter	EYC gm/liter
$Na_3C_6H_5O_7 \cdot 2H_2O$		28.0
N-tris-(Hydroxymethyl) methyl-2-aminoethane sulfonic acid (TES)	28.8	
Glucose or fructose	5.0	8.0
NaCl	0.14	
$NaH_2PO_4 \cdot HOH$	0.03	
KCL		
K_2NO_3	0.42	
$MgCl_2 \cdot 6HOH$	0.37	
$CaCl_2$	2.26	
Penicillin-G-Na	0.31	0.31
Sulfanilamide	1.50	1.50
Polymixin-B-SO_4	0.007	0.007
Egg Yolk	200.0	200.0
Catalase	75 IU/ml	75 IU/ml
Mycostatin	4 IU/ml	4 IU/ml

[a]According to C. E. Johnson, Jr., 1966, West Virginia, unpublished.

Experiment 2

Breeding at first estrus after treatment with progestins has been found to result in decreased fertility (Robinson, 1967; see also results of Experiment 1). Decreased fertility is temporary and second estrous periods are still grouped precisely (Edey and Thwaites, 1966; Moore *et al.*, 1967). Therefore, ewes were bred at second estrus after pessary removal in this experiment, with the expectation of higher fertility. The experiment was carried out in a flock of 195 blackfaced native ewes in August, 1969.

Sixteen days after pessary removal ewes were assigned at random to NM and placed in one of two groups of 32 ewes each) or to AI (128 ewes). Each group of ewes to receive NM was placed with four brisket-painted, fertile Hampshire rams and observed for estrus twice daily. Ewes to receive AI were kept with five painted vasectomized rams and observed for estrus twice daily. They were assigned to treatment and separated from the rams as they came into heat. Inseminations were made at 6 a.m. and 6 p.m.

Collected semen was diluted either in egg yolk fructose citrate (EYFC) with 10 mcg/ml. of β-amylase (1:10) and stored 12 hours at 5^{o} C or inseminated fresh and undiluted. Ewes received either 0.1 ml. of fresh, undiluted or 1 ml. of diluted semen (average of 698 X 10^{6} cells/insemination) as above.

RESULTS AND DISCUSSION

Experiment 1

No differences were found among the overall conception rates obtained with semen diluted in TEY with or without β-amylase or EYGC without β-amylase (Table 2). Higher conception rates were obtained from ewes bred with spermatozoa extended in EYGC with β-amylase, overall and in the cervix, than in all other ewes treated except NM ewes. Furthermore, no difference was found between NM ewes and ewes bred with spermatozoa in EYGC with β-amylase. These findings indicate a dependence upon diluent for the expression of a beneficial effect of β-amylase.

Experiment 2

Only 102 of the 195 ewes placed on experiment exhibited behavioral estrus, possibly because of the early breeding time (August) and/or because some ewes (not identified individually) were suckling lambs until 24 hours prior to the breeding time. At the conclusion of the breeding period only 61 percent (41 percent for lot 1 and 81 percent for lot 2) of the NM ewes and 49 percent of the AI group had been found in estrus (Table 3). Randomization of the ewes assigned to the NM groups was incomplete since ewes randomly assigned to lot 1 came from two of four pastures while those assigned to lot 2 came from two

TABLE 2

Effect of Addition of β-Amylase to TEY and EYGC Diluents on Conception Rates of Ewes Bred Artificially

Treatments	No. of Ewes at Lambing	No. of Ewes Lambing	No. of Lambs	Conception Rates Overall	Conception Rates Cervix
				%	%
NM	76	23	24	30.3[ac]	
EYGC	38	0	0	0.0	0.0 (26)[b]
TEY	41	1	1	2.4	4.3 (23)
EYGC + β-amylase	39	8	8	20.5[ac]	29.2 (24)[ac]
TEY + β-amylase	39	1	1	2.6	5.3 (19)

[a]Different at p 0.01 from overall conception rates of other treatment groups.

[b]Number of ewes inseminated in the os cervix in parentheses.

[c]Not different from each other at P< 0.05, but different from all other groups at p< 0.05

other pastures. As a result, all lactating ewes mated NM were in lot 1. However, since AI ewes were assigned at random from each pasture and were randomized to treatment as they were detected in estrus, the lactating ewes were randomly distributed among the four AI treatments.

The conception rate of the NM ewes in lot 1 (38.5 percent) was not different from that of any other group (P<0.05; Table 3). The ewes receiving fresh, undiluted semen had conception rates (60.1 and 78.6 percent) similar to those of the ewes mated to rams in lot 2 (73.1 percent). Ewes bred with semen diluted and stored in EYFC with β-amylase had lower conception rates (P<0.05) than those bred with fresh, undiluted semen or the NM ewes in lot 2.

The benefit derived from breeding at 12 hours versus immediately after detection of behavioral estrus was not significant. However, conception rates at 12 hours after detection versus zero time were 18 percentage points higher with fresh semen and nearly 12 percentage points higher (doubled) with the diluted, stored semen. These results support the recommendation of Terrill (1960) that if ewes are to be inseminated only once, it be done at 12 hours after onset of estrus at twice daily observation periods.

TABLE 3

Effect of Time of Insemination at Second Estrus after Treatment with Flurogestone Acetate on Conception Rates of Ewes Bred Artificially with Fresh, Undiluted Semen or Semen in EYGC + β-Amylase (10mcg/ml.)

Breeding Regime	Semen Volume (ml.)	Time of Insemination Post Estrus (hr.)	No. of Ewes Detected in Estrus and Inseminated	No. of Ewes Lambing	No. of Lambs	Conception rates
						%
Natural-lot 1	–	–	13	5	7	38.5[a]
Natural-lot 2	–	–	26	19	30	73.1
Fresh semen	0.1	0	15	9	15	60.1
Fresh semen	0.1	12	14	11	19	78.6
Diluted, stored semen[b]	1.0	0	17	2	3	11.8[a]
Diluted, stored semen	1.0	12	17	4	7	23.5[a]

[a]Not different at $p<0.05$.

CONCLUSION

The relative value of adding β-amylase to extenders for ram spermatozoa is not clear. When β-amylase was added at a concentration of 2 mcg/ml to semen in an egg yolk glucose citrate extender, conception rates of ewes bred AI were not different than those of ewes bred naturally at first estrus after progestin treatment. However, no beneficial effects of β-amylase on conception rates were observed when a TES-buffered egg yolk extender was utilized. At second estrus after progestin treatment, semen diluted in an egg yolk fructose citrate diluent was inferior to either natural mating or fresh, undiluted semen. Breeding at 12 hours after detection of estrus was not significantly superior to breeding immediately after estrus was detected.

ACKNOWLEDGMENTS

The authors appreciate the financial support for these studies from G. D. Searle and Company. The assistance of Dr. Lamont W. Smith, Mr. Edward L. Squires, Mr. Charles E. Cooke, and Mr. Ronald Metcalfe is gratefully acknowledged. Dr. Grover H. Phillippi and Messrs. John and Samuel Tuckwiller provided the ewe flocks.

LITERATURE CITED

Austin, C. R. 1951. Observations on the penetration of the sperm into the mammaliam egg. *Aust. J. Sci. Res.* 4:481.

Chang, M. E. 1951. Development of fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature* 168:697.

Dauzier, L., and C. Thibault. 1959. Données nouvelles sur la fécondation *in vitro* de l'oeuf la lapin et de la brebis. *C. R. Seance Acad. Sci.* 248:2655.

Edey, T. N. and C. J. Thwaites. 1966. Synchronization of ewes at the second estrus after progestogen treatment. *Proc. Aust. Soc. Animal Prod.* 6:30.

Fryer, H. C. 1966. *Concepts and Methods of Experimental Statistics.* Allyn and Bacon, Inc., Boston. p. 96.

Kirton, K. T., L. J. Boyd, and H. D. Hafs. 1968. Fertility of bull semen with added amylase. *J. Dairy Sci.* 51(9):1426.

Kirton, K. T., and H. D. Hafs. 1965. Sperm capacitation by uterine fluid or beta-amylase *in vitro*. *Science* 150:618.

Mattner, P. E. 1963. Capacitation of ram spermatozoa and penetration of the ovine egg. *Nature* 199:772.

Moore, N. W., T. D. Quinlivan, T. J. Robinson, and J. R. Smith. 1967. "The fertilization of ova following natural mating or artificial insemination after withdrawal of SC-9880-impregnated intravaginal sponges from cyclic merino ewes." In *The Control of the Ovarian Cycle in the Sheep.* T. J. Robinson, ed. Sydney University Press, p. 169.

Ozin, F. V. 1956. Artificial insemination—a valuable reserve for improving fine- and semifine-wooled sheep. *Ovcevodstvo.* 6:17 (*Animal Breed. Abstr.* 25, No. 233).

Robinson, T. J. 1967. "Conclusions." In *The Control of The Ovarian Cycle in Sheep.* Sydney University Press, p. 237.

Terrill, C. E. 1960. "Sheep and goats." In E. J. Perry, *Artificial Insemination of Farm Animals.* Rutgers University Press, New Brunswick, p. 178.

PART 3

EFFECT OF TIME OF INSEMINATION ON CONCEPTION RATES OF EWES BRED WITH FRESH, UNDILUTED SEMEN

J. B. Peters, Lance Kauf and E. K. Inskeep

An experiment was carried out on two cooperating farms in the Allegheny Highlands Project to compare two times for insemination in relation to the time of detection of heat. It is important to provide a maximum number of sperm which are capable of fertilizing the egg at the site of fertilization when the egg arrives at the site. This site has not been determined precisely in the ewe but it can be assumed that the egg will reach the site of fertilization shortly after ovulation. The time of ovulation and the time of insemination in relation to ovulation are therefore important in achieving maximum conception. Ovulation in the ewe has been shown to occur 21 to 26 hours after release of luteinizing hormone (Geschwind, 1972) which occurs near the onset of estrus. Parsons and Hunter (1967) checked heat every 4 hours and found that ovulation occurred at least 24 hours following the detection of heat. The two times selected to inseminate ewes in this study were approximately 12 and 24 hours following the detection of heat at twice daily heat checks.

MATERIALS AND METHODS

A total of 203 ewes on two farms were treated initially in preparation for this experiment, which was carried out in October and November, 1971. The ewes on Farm A (105) were primarily of Suffolk breeding except for 25 whitefaced Western ewes. The ewes on Farm B (98) were either crossbred ewes with Dorset, Hampshire and Shropshire blood or whitefaced Western ewes. Estrus and ovulation were controlled by inserting vaginal pessaries containing 20 mg. of flurogestone acetate for a period of 14 days. The first postreatment estrus was ignored and ewes were bred at the second heat, 17 to 19 days after pessary removal.

As ewes exhibited estrus, determined by the use of raddled vasectomized rams, they were assigned at random to one of three groups. Ewes in group 1 were inseminated approximately 12 hours after exhibition of estrus. Ewes in

group 2 were inseminated approximately 24 hours after exhibition of estrus. Ewes in group 3 were inseminated at both times. All inseminations were performed by the same inseminator on both farms.

Artificial insemination was performed using 0.1 ml. fresh, undiluted semen collected by artificial vagina just prior to use. A vaginal speculum was utilized to expose the cervix and the semen was deposited inside the os cervix. Inseminated ewes were turned into a separate pasture because fertile rams were turned out with ewes not exhibiting estrus immediately after the AI period. This practice insured that ewes exhibiting estrus after the AI period would have an opportunity to be bred and also that ewes which had been artificially inseminated and were still receptive to the ram would not be bred naturally.

Data were analyzed for effects of farm, breeding time (group) and time of day that the ewes came in heat by the method of Brain (1971).

RESULTS AND DISCUSSION

Seventy-four of 105 ewes on Farm A and 80 of 98 on Farm B exhibited heat during the AI period (days 17, 18 and 19 after pessary removal). Sixty-five ewes on Farm A and 72 ewes on Farm B were bred artificially. One ewe in group 1 and two ewes in group 2 from Farm A died before lambing time and were deleted from the data. The remaining ewes exhibiting estrus were hand mated as they came into heat.

Conception rate and prolificacy by group for the individual farms and overall are presented in Table 1. There was an apparently lower conception rate in groups 1 and 2 than in group 3, particularly on Farm B. This trend, although not significant with these small numbers, indicates a variation between ewes as to the optimum time of insemination. Checking heat at 12-hour intervals allows considerable variation to exist between the actual onset of heat and the time of heat detection. Therefore insemination at 12 and 24 hours after detection of heat would result in decreased variation in the relation of the timing of insemination to the occurrence of ovulation. The use of double insemination should allow sperm to be present at the site of fertilization at more nearly the correct time.

The data (Table 1) also show that breeding group had no effect on lambs born per ewe lambing. There was a large and highly significant difference between farms in the lamb crop, however. Ewes on Farm A dropped an average of 1.67 lambs while those on Farm B dropped only 1.35.

In Table 2 the data have been separated to show the effects of the time heat was first observed on conception. There was a significant interaction of breeding group with time of onset of heat and an interaction of farm with group and time of heat. The results indicate that insemination at 24 hours as in group 2 was

TABLE 1

Conception and Lamb Crop Data

Farm	Group*	No. Ewes Bred	No. Ewes Conceived	No. Lambs Born	Lambs Born Per Ewe Lambing
A	1	19	12 (63%)	20	1.66
	2	19	13 (68%)	20	1.53
	3	24	17 (71%)	30	1.75
B	1	29	20 (69%)	27	1.35
	2	22	17 (77%)	23	1.35
	3	21	20 (95%)	27	1.35
Both	1	48	32 (67%)	47	1.47
	2	41	30 (73%)	43	1.43
	3	45	37 (82%)	57	1.54
All		134	99 (73%)	147	1.48

*1–Bred 12 hours after observed heat.

2–Bred 24 hours after observed heat.

3–Bred 12 and 24 hours after observed heat.

more effective when ewes were first seen in heat in the evening whereas there was a small advantage to inseminating at 12 hours when ewes first exhibited estrus in the morning. However, inseminating at both 12 and 24 hours was equally as effective or more so than at either time alone except for ewes coming in heat in the evening on Farm A. When ewes came in heat in the evening, waiting 24 hours to inseminate was on the average 30 percentage points more effective than breeding the next morning. For ewes in heat in the morning results were 13 percentage points better by breeding the same evening. Thus it is tempting to suggest that all ewes should be bred in the evening. Small numbers of ewes and variation in data such as these allow only speculation as to concrete findings. However, an explanation for the apparent difference between the

TABLE 2

Effect of Times of Heat and Breeding on Conception Rate

Time of Heat	Farm	Breeding Group 1 No. of Ewes In Heat	1 Conceived[a]	1 % Conceived	Breeding Group 2 No. of Ewes In Heat	2 Conceived	2 % Conceived	Breeding Group 3 No. of Ewes In Heat	3 Conceived	3 % Conceived
A.M.	A	13	8	62	8	4	50	11	11	100
	B	20	15	75	13	8	62	10	9	90
	Both	33	23	70	21	12	57	21	20	95
P.M.	A	6	4	67	11	9	82	13	6	46
	B	9	5	56	9	9	100	11	11	100
	Both	15	9	60	20	18	90	24	17	71

[a]Conception is defined by birth of one or more lambs 143 to 151 days post mating.

results for ewes with morning and evening onset of heat can be offered. In actual practice morning heat checks were made at 7 a.m. and evening checks were made around 5 p.m. The length of time from the evening check until the next morning was actually longer (around 14 hours) than the length of time from the morning check until the next evening (around 10 hours). The ewes exhibiting heat in the morning thus would have had more variation in the length of time from initiation of heat until heat was detected or until insemination than ewes in heat in the evening. Thus the double insemination should make sperm available at the correct time more often. This was the case since 95 percent of the ewes which came in heat in the morning settled to two inseminations.

The results of this study indicate that a single artificial insemination with fresh semen in the ewe can yield acceptable conception rates at the second heat period after synchronization of estrus. Limitations to AI include the difficulty in collecting and processing semen and in the identification of truly superior sires by performance testing. At the present time AI in sheep is time-consuming and is practiced only on a demonstrational basis. This study suggests the possibility that insemination once a day, in the evening, might be a practical means to reduce labor. This possibility is worthy of further investigation.

ACKNOWLEDGMENTS

The support of the Allegheny Highlands Project by The Rockefeller Foundation is gratefully acknowledged. We thank Maxilyn Teter and Darley Ware for the use of their ewe flocks and G. D. Searle and Co. for Cronolone sponges.

LITERATURE CITED

Brain, Carlos W. 1971. The exact partition of the total Chi-square of bionomial data. Problem Report. Division of Statistics, West Virginia University, Morgantown.

Geschwind, I. I. 1972. Dynamics of pituitary gonadotropin secretion. *J. Animal Sci.* 34 (Suppl. I):19.

Parsons, S. D. and G. L. Hunter. 1967. Effect of the ram on duration of oestrus in the ewe. *J. Reprod. Fert.* 14:61.

[Blank Page in Original Bulletin]